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human endothelial cells HUVEC. Lastly, four intraperitoneal injections of scFv JH1 (100 µg/injection) on day 9, 13, 17 and 19 following transplant of human ovarian carcinoma cells MA148 to nude mice (2x10⁶ cells/mouse) significantly inhibited tumor growth. These results suggest that anti-VEGF scFv JH1 may be a useful reagent to develop anti-angiogenic therapy of solid tumors.

#4802 Surface plasmon resonance-based competition assay to assess the sera reactivity of variants of humanized antibodies. Noreen R. Gonzales, Peter Schuck, Jeffrey Schiom, and Syed V. S. Kashmini. *LTIB, CCR, NCI, NIH, Bethesda, MD, and MIR, DBEPS, ORS, NIH, Bethesda, MD.*

To evaluate the relative potential immunogenicity of variants to the parental humanized antibody (Ab), we have taken the approach of comparing the reactivities of the humanized Ab and its variants to patients' sera containing anti-idiotypic antibodies (anti-Ids) to the parental Ab. Sera reactivity is measured by the ability of a humanized Ab variant to compete with the parental Ab for binding to patients' sera. We developed a Surface Plasmon Resonance-based assay to monitor the binding of the sera anti-Ids to the parental Ab and the inhibition of this binding by the variants. This new assay requires no radiolabeling, is relatively less time-consuming, and uses only small amounts of serum through an innovative sample application technique. To validate the assay, we have tested the relative reactivities of the CDR-grafted anti-carcinoma Ab, HuCC49, and two variants, designated V5 and V10, to the sera of two patients, who were earlier administered radiolabeled murine CC49 in a clinical trial. IC₅₀s, the concentrations of the competitor antibody required for 50% inhibition of the binding of the serum to HuCC49, showed a 9.6-fold reactivity difference between HuCC49 and V10 to one serum, and a 164-fold difference in their reactivities to the other serum. The results may indicate the difference between the potential immunogenicities of the variants and the parental humanized Ab. Furthermore, the assay can be adapted to allow a comparison of relative amounts of anti-Ids present in sera of different patients without removing the circulating antigen. This can facilitate the rapid screening of the sera of patients involved in clinical trials for the presence of anti-Ids.

#4803 Characterization of five new fully human monoclonal IgM antibodies isolated from carcinoma patients. Stephanie Braendlein, Frank Hensel, Judith Lorenz, Matthias Eck, Bertram Illert, Justus Mueller, Hans Konrad Mueller-Hermelink, and H. Peter Vollmers. *Pathology, Univ. Wuerzburg, Wuerzburg, Germany, and Surgery, Univ. Wuerzburg, Wuerzburg, Germany.*

Monoclonal antibodies are accepted to be ideal adjuvant therapeutical reagents for all kinds of diseases. Polyvalent (crosslinking) and low mutated IgM antibodies (less immunogenic) are believed to be the most effective weapons against cancer. The best source for these types of antibodies is the cancer patient itself. Using conventional hybridoma technique, not only fully human monoclonal IgM antibodies are isolated, but also new targets are identified by the same experimental approach. The resulting antibodies can be used directly for therapeutical purposes without further modulation and manipulation. B-cells from patients with carcinomas of colon, pancreas and lung were isolated from lymph nodes and immortalized by fusion to the heteromyseloma HAB-1/X. Resulting human monoclonal antibodies were tested initially on autologous tumor tissue and tumor reacting antibodies were further tested on panels of malignant and healthy tissues to determine the specificity. Antibodies were blotted on cell extracts to characterize the targets and tested in functional assays for cytostatic/toxic activities. We have generated and characterized five new human monoclonal IgMs. The mainly germ-line coded IgM antibodies CM-1 and CM-2 (colon), PM-1 and PM-2 (pancreas) and LM-1 (lung) are specific for malignant tissues and show only restricted reactivity with healthy cells. Biochemical analysis to determine the corresponding receptors are underway. Tested for functional *in vitro* activity, human antibody CM-1 inhibits tumor cell proliferation by inducing apoptosis. Adjuvant treatment of cancer with monoclonal antibodies requires new antibodies and new targets. By using classical human hybridoma technique we have established five new human monoclonal IgM antibodies from patients with different cancers. All five antibodies show a tumor restricted reactivity pattern and at least one antibody (CM-1) induces apoptosis. This shows that carcinoma patients have an anti tumor B-cell immunity and manifest tumors are not a matter of quality but most likely of quantity of humoral immunity.

#4804 Granulocyte-colony stimulating factor enhances chimeric antibody Nd2 dependent cytotoxicity against pancreatic cancer mediated by polymorphonuclear neutrophils. Yutaka Tamamori, Tetsuji Sawada, Tamahiro Nishihara, Yoshito Yamashita, Masaichi Ohira, and Kosei Hirakawa. *Department of Surgical Oncology, Osaka City University Graduate School of Medicine, Osaka, Japan.*

Nd2 is a monoclonal antibody against pancreatic cancer. We have previously reported that human/mouse chimeric antibody Nd2 (c-Nd2) can induce antibody-dependent cell-mediated cytotoxicity (ADCC) with peripheral blood mononuclear cells (PBMs) as effectors. In this study, we investigated c-Nd2 induced ADCC by polymorphonuclear neutrophils (PMNs) as effector cells and the effects of G-CSF in enhancing this cytotoxicity. Cytotoxicities for pancreatic cancer cell line SW1990 were dose-dependently increased during mixed PMN and tumor cell culture with c-Nd2, and these cytotoxicities were significantly suppressed by neutralizing antibodies against CD16, which were Fcγ receptors expressed on PMN membranes. Furthermore, the treatment of PMNs with G-CSF was significantly

enhanced. *In vitro* c-Nd2 induced ADCC activity with these PMNs. The tumor growth of SW1990 subcutaneously transplanted nude mouse tended to be suppressed by i.p. administration of c-Nd2 or G-CSF. In addition, the combination of c-Nd2 and G-CSF significantly inhibited this *in vivo* tumor growth, which was accompanied by a strong infiltration of PMNs into and around the transplanted tumor, as confirmed by immunohistochemical study with anti-mouse neutrophil elastase antibody. These results suggest that PMNs play an important role in c-Nd2 induced ADCC and that combination immunotherapy of c-Nd2 with G-CSF may be beneficial in clinical applications against pancreatic cancer by enhancing ADCC induced by PMNs.

#4805 Development of a single chain Fv from a human anti-ID monoclonal antibody that mimics the GD2 antigen: A vaccine candidate for cancer immunotherapy. Pradip K. Maiti, Joycelyn Entwistle, Danen Fast, and Glen MacDonald. *Viventia Biotech Inc, Winnipeg, MB, Canada.*

We have generated a human anti-id antibody namely 4B5, that mimics the GD2 ganglioside, a tumor antigen that is overexpressed on melanoma, neuroblastoma and small cell lung cancers. In addition to an intact IgG antibody, we have also developed a single chain Fv form. The specificity of the 4B5 scFv was demonstrated by its ability to bind to 14G2a, an anti-GD2 MAb, and to inhibit the binding of 14G2a to a GD2-positive melanoma cell line SK-MEL-5. To explore the potential of 4B5 as an anti-id cancer vaccine, we have assessed and compared the immunogenicity of both the scFv and the intact IgG forms of 4B5 in mice. Immunization with either form of 4B5 resulted in the induction of both humoral and cell-mediated immune responses. However, mice immunized with the 4B5 scFv demonstrated a stronger humoral immune response. This was indicated by a higher 4B5-specific antibody titer, a stronger anti-anti-id response, and a stronger anti-GD2 antibody (Ab3) response. A somewhat stronger cell-mediated immune response was induced in mice immunized with the 4B5 scFv, as demonstrated by the production of IL-2, IL-13 and IFN γ in the primary culture of spleen cells. These results indicate that the scFv form induces a stronger anti-id response than the IgG. Thus, the 4B5 scFv has potential as an anti-id vaccine for cancer immunotherapy.

#4806 Cancer autoantigens are cleaved by granzyme B: A potential mechanism for revelation of cryptic epitopes. Danielle Ulanet, Amy Cox, Chi V. Dang, Livia Casciola-Rosen, and Antony Rosen. *Johns Hopkins University School of Medicine, Baltimore, MD.*

The ongoing identification of tumor antigens has begun to elucidate that the majority of these proteins are non-mutated and thus immune responses against them represent an autoimmune phenomenon. Unlike in autoimmune disease, where the immune response against self proteins is detrimental to the host, immune responses against self proteins in tumors have been associated with a more favorable prognosis. Thus, the identification of a common mechanism responsible for rendering such self proteins immunogenic, may greatly benefit the field of tumor immunology. The recent finding that the majority of autoantigens targeted across the spectrum of systemic autoimmune diseases are selectively cleaved by the cytotoxic lymphocyte granule protease granzyme B to generate unique fragments, implies that altered cleavage of these molecules, during cytotoxic lymphocyte-granule induced cell death, may play a role in specifically breaking tolerance to them. We demonstrate here that susceptibility to specific cleavage by granzyme B is also a feature shared by cancer autoantigens. The molecules examined (tyrosinase, c-myc, nucleophosmin/B23, UBF/NOF-90, and fibrinogen) included autoantigens of diverse function and sub-cellular localization and were targeted by autoantibodies in various different malignancies. We propose that altered cleavage of these molecules in the pro-immune setting of cytotoxic lymphocyte induced cell death, combined with increased expression in tumors, may play a role in inciting the specific adaptive immune response in cancer.

#4807 Mutagenesis of human RNase for immunotoxin therapy. Heidi A. Erickson, Michelle Jund, and Christopher A. Pennell. *University of Minnesota, Minneapolis, MN.*

An immunotoxin is a chimeric molecule consisting of a targeting moiety and a toxin moiety. In the treatment of T cell acute lymphoblastic leukemia (T-ALL), immunotoxins directed against CD7, an antigen highly expressed on leukemic T cells, have been constructed. Historically, the toxin moiety has been plant or bacterially derived; however, their efficacy in an immunotoxin is limited by immunogenicity and non-specific toxicity. An appealing alternative to such toxins is to use a human toxin such as ribonuclease (hRNase) in an immunotoxin construct. RNases are efficient enzymes that digest RNA, prohibiting protein synthesis thereby leading to apoptosis. Normally, they are efficiently inhibited by ribonuclease inhibitor (RI), a cytosolic protein found in abundance in most cells. If hRNase was mutated such that it was bound less effectively by RI, it could be used to derive a potent, less immunogenic immunotoxin. To this end, a series of mutations were made within key regions in the hRNase interfacial e. These mutants will be examined for their ability to evade RI binding and linked to a targeting moiety directed against CD7.